

Microbial Contamination of Food Available in Sub Metropolitan City Birgunj in Nepal and Its Effect on Human Health

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ABSTRACT:

An epidemiological study on food-borne pathogens and bacterial infection etiology of road side food stalls and slaughtering houses in sub metropolitan city Birgunj, Nepal was performed. The objective of this study was to identify and evaluate the food-borne bacterial agents present in several food products and analyze the real cause of misleading human safety. To attain our objective twenty five food handlers were interviewed using a semi-structured researcher-administered questionnaire, most of them reported diarrhoea, vomiting, abdominal pain, and fever may be caused by food contaminations. Microbiological analysis of 48 samples of ready-to-eat food revealed the presence of *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Salmonella typhi*, *Shigella sonnei*, and *Pseudomonas aeruginosa* contamination in most of them. *E. coli*, *Salmonella* and *Shigella* were recovered from various samples of mutton and chicken. The prevalence of food contamination in the food stalls was found due to lack of knowledge at one hand but negligence of food handlers on the other hand. Those food handlers who know the disease associated with food contamination in the interview, food samples from their stalls also showed positive result in food contamination shows their negligence. Maximum bacterial load (58×10^6 CFU/g) was seen in that area (Kumhal tole) where the rate of sale is slow, whereas minimum load (360 CFU/g) in fast rate of sale ie Meena bazaar. Various types of pathogenic bacteria may contaminate food have been reported time to time in literature from many parts of the world. We find in the present study that training of food safety to food handlers and strictly implementation of hygiene condition by the government is the present demand in the sub metropolitan city Birgunj, Nepal.

Keywords: Food contamination, food stalls, food-borne illnesses, parasites, bacteriology, food industry.

INTRODUCTION

Food contamination is the introduction or occurrence of a contaminant (any biological or chemical agent, foreign matter or other substance not intentionally added to food which may compromise food safety or suitability) in food or a food environment [1]. Food is prone to contamination at every stage in the food chain, i.e. the various stages from primary production of food to when it is ready for consumption. The consumption of food contaminated by micro-organisms will result in food-borne illnesses. These are usually either infectious or toxic in nature, caused by agents that enter the body through ingestion of food [2]. The main food-borne pathogens that can contaminate food include, but are not limited to, *Salmonella* spp, *Staphylococcus aureus*, *Vibrio cholerae* or other *Vibrio* spp, *Clostridium botulinum*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas* spp, while the intestinal parasites include *Giardia lamblia*, *Entamoeba histolytica*, *Ascaris lumbricoides*, *Trichuris trichiuria* and Hookworm spp. [3, 4]. Food-borne illnesses are major international health problems and important causes of reduced economic growth [5]. They not only significantly affect people's health and wellbeing, but also have economic consequences for

individuals, families, communities, the food industry and the national economy [6, 7]. Up to one-third of the population of developed countries are affected by food-borne illnesses each year. The problem is even more widespread in developing countries, where the poor are most susceptible to illness and death. Food- and water-borne diarrheal diseases are the leading causes of illness and death in developing countries, killing an estimated 2.2 million people annually, most of them are children [8]. Although diarrhea is the most common symptom of food-borne illness, other serious consequences include kidney and liver failure, brain and neural disorders, and death [9]. Food-borne illnesses have continued to form a significant part of the morbidity and mortality of Nepalese, and have been on the increase in recent times. The massive proliferation of fast food restaurants in Birgunj since the beginning of this decade have resulted in a corresponding increase in fast food consumption. The contamination of these fast foods would lead to a high incidence of food-borne illnesses in the city. Thus, the aim of this study was to identify factors affecting food contamination and to determine the prevalence of food contamination in the food available in sub

metropolitan city Birgunj, Parsa district, Nepal in order to ascertain the safety of these foods.

Food contamination is the results of eating organisms or toxins in contaminated food. Most cases of food contamination are from common bacteria like *Staphylococcus* and/or *E. coli*. The microbial contamination is a universal problem due to the presence of microbial propagation in the environment. The different types of microbes like – thermophilic, thermoduric and psychrophilic bacteria are present in the environment either in active condition or in dormant condition. When microbes enter food from soil, water, air, etc. they may either cause it to spoil or make it dangerous to eat or enhance it to another form i.e. still acceptable as food. On the basis of spoilage they can be categorized as perishable, semi perishable and/or non-perishable food.

One can try to keep the contamination to a minimum by cleaning the hand, processing equipments and utensils. Water and air is used for cleaning and food ingredients. Microbes of air, water and soil can easily contaminate the processing area, food and processed food. The possibility of contamination from atmosphere, soil or water is high. Food contamination can affect one person or it can occur as an outbreak in a group of people who all eat the same contaminated food. Even though food contamination is common in India, Nepal, Bangladesh, Pakistan and it affects approx. 60-80 million people worldwide each year and results in approximately 06-08 million deaths. Intake of spoiled or contaminated food cause food-borne illness and food poisoning to consumers. In developing countries most of the fatal diseases are due to consumption of contaminated food. We live in a microbial world and there are several opportunities for food to be contaminated during the processing of foods.

Freshly eaten vegetables constitute an ordinary part of the diet of many people. Food-borne parasitic infections may be associated with consumption of these contaminated fresh items. Fruits and vegetables, particularly those eaten raw and without peeling, have been demonstrated to be the vehicle for transmission of a range of parasites [9]. Improperly washed vegetables represent a major risk factor and mode of parasite transmission. Contamination could be due to several factors including the use of untreated wastewater and water contaminated with sewage for irrigation as well as handling vegetables

with contaminated hands. Most people are not aware of the danger to their health posed by parasites nor do they know from where and how parasites are acquired [9].

The food borne illness such as diarrhea, vomiting, fever, sore throat with fever and jaundice has been reported time to time may be caused by bacterial contamination. An outbreak of food-borne illness occurs when a group of people consume the same contaminated food. It may be a group that ate meal together somewhere or it may be a group of people who do not know each other at all but who all buy and eat the same contaminated food items from food stalls or a restaurant. For an outbreak to occur something must have happened to contaminate a batch of food that was eaten by a group of people. A food-borne outbreak is an indication of malfunctioning of food safety system. The full investigation of such outbreaks requires a team with multiple talents, including the epidemiologists, microbiologists, food sanitarians, veterinarians and factory process engineers.

MATERIALS AND METHODS

Preparation of samples

A total of 30 raw food samples including fruits, vegetables, chicken, mutton and 48 ready-to-eat food samples such as salad, fried rice, cured meat, fried fish, chicken-chilli, matar-paneer (pea-cheese) etc. were collected aseptically from road-side stalls of sub metropolitan city Birgunj, Nepal. An attempt was undertaken to interview the food handlers about their knowledge of health and hygiene and the symptoms of diseases associated with food contamination using a semi-structured researcher-administered questionnaire. Almost 25 food handlers showed interest and other refused. Simultaneously food samples were scooped with a sterile spatula into a sterile bottle or polythene bag containing alkaline peptone water, and transported to microbiology laboratory of Hari Khetan Multiple Campus, Birgunj, Nepal within 1h of collection after proper labeling their name and place using a permanent marker.

Microbiological analysis

Collected vegetables were soaked and then washed by vigorous shaking with a suitable quantity of sterile normal saline solution. Washed sample was transferred into sterile conical plastic tubes along with normal saline, and centrifuged at 2000 rpm for 15 minutes. Then, the supernatant was discarded carefully, and the sediment was transferred to

microscope slides for parasitological examination under light microscope (x100–400 magnification). Normal saline and iodine preparations of the samples were examined for parasitic protozoa and helminths in the laboratory for half of the samples. Other half of the samples were serially diluted in normal saline up to 10^{-5} dilution and uniformly spread over media plates of blood agar, chocolate agar and Mac-Conkey agar to get clear evidence of microbes present in food samples. Aseptic techniques were maintained during media preparation, collection, transportation and analysis of samples; the media plates were checked thoroughly before use; and the temperature of the incubator was maintained at 37°C throughout the inoculation period. Samples spread plates were incubated at 37°C for 18-24h in BOD incubator. Colony forming units (CFU) were counted and recorded after proper growth. Biochemical tests as per the standard protocols were carried out on the

next day and onward that yielded growth. Gram staining was also performed to differentiate Gram-positive and Gram-negative bacteria. Similarly oxidase test was performed on Gram-negative bacilli and those that were sub cultured on nutrient agar while all Gram-positive cocci that were catalase positive were sub cultured on mannitol salt agar. Catalase and coagulase tests were performed on Gram-positive cocci, and motility tests were undertaken on both Gram-positive and Gram-negative bacilli. This was followed by inoculation of the Gram-negative bacilli in glucose, maltose, sucrose, lactose, triple sugars, peptone water broth (for indole test) and other media such as urea and citrate. Isolated bacteria were identified by biochemical characterization to follow the protocols of Bergey's manual of determinative bacteriology [10].

Table 1: Symptoms of disease caused by the consumption of contaminated food

Symptoms	Frequency (n = 25)	%
Diarrhea	18	72
Abdominal pain	11	44
Vomiting	19	76
Fever	8	32

Data is based on interview by various respondents' food handlers

Table 2: Number of total CFU/g present in non-veg food items collected from various locations.

S.No.	Sample	Location	Total CFU/g	<i>E.coli</i>	<i>Salmonella</i>	<i>Shigella</i>
1.	Mutton	Meena Bazaar	360	++	-	-
2.	Chicken	Maisthan	600	++	++	-
3.	Chicken	Birta	50×10^5	++	++	-
4.	Chicken	Chhapkaiya	35×10^6	-	-	++
5.	Mutton	Shreepur	15×10^3	-	-	-
6.	Chicken	Ranighat	60×10^5	++	++	-
7.	Chicken	Nagwa	40×10^5	++	-	-
8.	Mutton	Chhapkaiya	38×10^5	++	-	-
9.	Chicken	Kumhal Tole	58×10^6	++	++	-
10.	Chicken	Panitanki	36×10^6	++	++	-

++, Positive; -, negative

Table 3: Data of bacteria found in ready to eat foods collected from various food stalls.

Type of food	Bacteria isolated*						Total
	Bc	Sa	Stm	Stp	Ss	Pa	
Salad	6	5	7	5	7	5	35
Fried rice	5	4	2	3	5	2	21
Cured meat	2	7	6	4	3	9	31
Fried fish	4	4	3	2	1	7	21
Chicken-chilli	1	4	1	2	7	4	19
Matar-paneer (Pea-cheese)	3	2	2	7	2	3	19

* Bc- *Bacillus cereus*, Sa- *Staphylococcus aureus*, Stm- *Salmonella typhimurium*, Stp- *Salmonella typhi*, Ss- *Shigella sonnei*, Pa- *Pseudomonas aeruginosa*

RESULT AND DISCUSSIONS

The data collected from the interview with responded food handlers are shown in Table 1. It is evident with the table that they know that various diseases are caused by food contamination, if micro-organisms contaminate food. Most of them mentioned bacteria, fungi and protozoans may contaminate these foods, whereas several of them knew the symptoms associated with microorganisms contaminated food consumption also. Diarrhea was mentioned by 18 respondents, abdominal pain by 11 respondents, vomiting by 19 respondents and fever was mentioned by 8 respondents.

Out of 30 samples examined, 24 were found contaminated with parasites and rest 6 was free from any microorganism. The data shown in table-2 shows that maximum CFU/g was recorded in chicken sample of kumhal tole (58×10^6), whereas, minimum in mutton sample of Meena bazaar (360). *E. coli* was observed in almost all samples except the samples of chicken of Chapkaiya and mutton of Shreepur. *Salmonella* was observed in 50% of the samples and *Shigella* was observed in only chicken sample of Chapkaiya (table-2).

Altogether, 48 samples of ready-to-eat foods including salad, fried rice, cured meat, fried fish, chicken-chilli, matar-paneer (pea-cheese) were examined for the presence of bacterial population. Out of 48 samples examined, 39 samples showed bacterial growth and 9 showed negative results. It is evident with the data shown in Table 3 that the majorities of isolates (35) were found in salad, while 31 in cured meat, 21 isolates in fried rice and fried fish and 19 isolates each in chicken-chilli and matar-paneer (pea-cheese). Various types of food was examined, that showed presence of *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Salmonella typhi*, *Shigella sonnei*, and *Pseudomonas aeruginosa*, but their number varied in different samples. The data shown in table-3 clearly shows that *Bacillus cereus*, *Salmonella typhimurium* and *Salmonella typhi* were isolated from 21 food samples each, *S. aureus* from 26 food samples, *Shigella sonnei* from 23 samples, and *Pseudomonas aeruginosa* were isolated from 30 food samples.

Evaluating protozoan and helminthic parasites, we observed *Entamoeba histolytica*, *Entamoeba coli*, and *Giardia lamblia* from contaminated vegetables and fruits; but, no any helminthic parasite species was recovered in the present study (Data not shown). The

pathogenic bacteria are destructive in nature which is resulting in the spoilage of food but still people unaware of all this consume the spoiled food and are in catch of disease. Sometime the spoilage cannot be distinguished and in such case people become prone to disease after consuming that food. This study showed that less than half of the food handlers had received training in food hygiene and safety. This underscores the Food and Agriculture Organization of the United Nations/ World Health Organization (FAO / WHO) recommendation that food handlers should receive training before starting work in any food establishment, with periodic refresher training thereafter. This may be due to ignorance of the management of fast food restaurants and different food stalls regarding such training programs, or may be because they do not wish to spend money on such training. The FAO/WHO recommendations emphasize the importance of personal hygiene of food handlers, and good food hygiene and safety practices with medical examinations carried out on food handlers if clinically and epidemiologically indicated [1]. It would be useful, especially in a developing nation such as Nepal where hygiene standards are poor; to know the baseline health status of food handlers before they commence work so that those found to be harboring any micro-organisms can be treated. Although the majority of the food handlers knew the symptoms of eating foods contaminated by micro-organisms, very few knew the specific names of micro-organisms that can contaminate food. A similar result was seen in a study in Iran, [11] but a study in Italy [3] found that almost half of the food handlers (48.7%) knew the names of micro-organisms that can contaminate food. There was a high prevalence of food contamination in the food restaurants studied. This result showed accordance to the studies in Accra, Ghana [8] and Ethiopia [12] which showed that street foods were heavily contaminated with micro-organisms, but far higher than a study in London which found a prevalence of food contamination of ready-to-eat cooked rice of 3% only [13]. The high prevalence found in the present study is a potential threat to the consumption of foods as this could lead to outbreak of food-borne illnesses.

The most commonly contaminated foods were salad and meat. This could be due to the fact that these foods require direct hand contact, and thus could be contaminated by the bare hands of the handlers during their preparation. Salad is prepared from raw vegetables which are at risk of contamination by soil micro-organisms. Moreover, heat is not applied

during salad preparation (heating kills some bacteria). A high rate of contamination of salad also arises if it is stored for a long period of time in inadequate temperatures, because this enables the micro-organisms to multiply. The mixing of some of the salad with salad cream has been found to constitute a good culture medium for bacteria; especially *S. aureus* [14]. Vegetable salad with mayonnaise was one of the foods implicated in a food-borne outbreak of *S. aureus* in Brodowski, Brazil [14]. *S. aureus* was isolated from all types of food samples, the majority of isolates were found in salad showed accordance with the previous report [15]. Food handlers can easily contaminate food with *S. aureus* if they sneeze or cough during food preparation, and if they do not wash their hands properly after making contact with their nose or after using the rest room. Although *B. cereus* was isolated from all types of food sampled, the majority of isolates was found in fried rice and salad. This was not surprising because the two distinct forms of food poisoning (emetic and diarrheal) caused by *B. cereus* are associated with fried rice [16]. *S. typhimurium* was isolated from salad and *S. typhi* was isolated from salad and fish. This was similar to the study in Ethiopia which showed that most street foods were contaminated with *Salmonella* spp. and *Shigella* spp [12]. This carrier state for *Salmonella* spp. and poor personal hygiene of food handlers could be responsible for the contamination of food by *Salmonella* organisms. This could also lead to a serious outbreak of enteric fevers and salmonellosis among individuals who patronize these food stalls.

This study showed that less than half of the food handlers had received training in food hygiene and safety, whereas trained food handlers show negligence to maintain hygienic condition. This underscores the Food and Agriculture Organization of the United Nations / World Health Organization (FAO / WHO) recommend that food handlers should receive training before starting work in any food establishment, with periodic refresher training thereafter. The government must provide training to such food handlers so that they could gain the proper knowledge of microbial contamination and can preserve food properly without any contamination. We recommend here to control such types of microbial load in highly nutritious & most energetic food like meat should be controlled by educating food handlers before starting their job and refresher training should be provided time to time. To minimize the food contamination, Fresh fruits,

vegetables and other foods should be preserved in lower temperature (eg. freeze or deep freeze) that micro-organisms may not grow easily and ready-to-eat food should be preserved either in hot conditions (eg. hot air oven) or in cold condition (eg. freeze). In addition to proper preservation, proper cleaning of hands by detergents is essential at the time of serving or eating foods. Slaughtering houses should be properly managed adopting standard procedure for slaughtering. Pure water and other essential equipments should be made available. Animals should be examined properly before slaughtering. Animals suffering from tuberculosis like disease should be prohibited for slaughtering. Meat act was formulated in 1991 to control the quality of meat by the government of Nepal that should be strongly implemented. Finger and nails of food handlers should be properly cleaned and they should encourage wearing clean protective clothing in food processing areas. They shouldn't spit, sneeze or cough near food items. Proper equipments should be designed to protect the food from physical contaminations. Cleaning and use of disinfectants should be done time to time. In conclusion, strictly implementation of laws in this issue and monitoring by the government agencies is most essential to attain healthy and hygienic society.

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DECLARATION FOR CONTRIBUTION OF WORKS

This research work has been completed by equal efforts of all the authors without any conflict of interest. Ankita has completed all the fields and laboratory works starting from the sample collection up to the data interpretations under round the clock guidance and supervision of Bisheswar Prasad Yadav. Umesh Prasad Shrivastava has designed the work plan in the beginning and prepared the manuscript at the last.

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